OFFICE OF NAVAL RESEARCH

FINAL REPORT

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TITLE OF PROJECT: Genetics of laboratory populations of Drosophila.

Objectives:

1. Study of the adaptive value of specific genes and the roles of natural selection and genetic drift in determining their frequencies.
2. Clarification of the role of sexual isolation and selective mating as evolutionary forces.
3. Study of the development of DDT resistance in experimental populations of Drosophila.
4. An analysis of the genetic and environmental factors causing the development of amelanotic tumor in Drosophila.

SUMMARY OF RESULTS:

A. Adaptive value of specific genes.

The experiments to test the adaptive values of specific genes have involved sex-linked and autosomal recessives and sex-linked and autosomal dominants in competition with their wild type alleles in population bottles. Furthermore, four mutants in the white series have been tested in competition with white itself in population bottles, and the sex-linked recessives, yellow, white, raspberry, and forked, and the autosomal recessive, glass, have been studied in competition with their respective wild type alleles in homeopathic vials supporting numerically small populations.

The results of the experiments with the very small populations with y, w, ras, f, or gl have been published in "Evolution". The following points emerged from these studies. The populations fluctuated in size from approximately 10 to 100 adults. Even in such small populations, selection favoring the wild type alleles was clearly at work, for, except in forked populations, the frequency of phenotypically wild type flies rose quickly to above 90% and remained there, indicating the phenotypic control of the populations by natural selection. Both the f and gl genes remained quite frequent, f because of its good viability, gl because carried in the heterozygous condition in both sexes. In addition to the effects of natural selection, the effects of random fluctuations in gene frequency were observed in these populations. As a result of these fluctuations, the individual populations came to differ widely from each other in gene frequency. In no instance, however, did the recessive mutant become fixed in the population. In some cases, the wild type genes became fixed, but other populations remained heterozygous so that these populations came to differ qualitatively from each other because of genetic drift. The effects of selection and genetic drift together produced the results obtained. Selection in favor of the wild type operated at all population sizes and apparently prevented the fixation of the deleterious recessives in any of the populations. However, random loss of the recessive did occur. When fluctuations in size occur, natural selection favors the wild type alleles while the population is relatively large. When the size is at a minimum, the wild type genes are already more frequent and selection still favors them. In addition genetic drift now reinforces the action of selection since it generally tends to reduce the frequency of the less frequent allele in a population. Thus, homozygosity for the favored alleles was achieved more rapidly in these small populations than it was in larger comparable populations because of the combined effects of selection and genetic drift.

The results with the sex-linked and autosomal recessives in competition with their wild type alleles at initially low frequencies in population bottles were included in the paper entitled "Competition between mutants in experimental populations of Drosophila melanogaster", which has been submitted for publication, and were also summarized in the Annual Progress Report for 1954. Briefly, certain significant features may be mentioned. The decline in frequency of the autosomal recessives, vestigial and glass, conformed to the theoretical curve expected for complete selection against an autosomal recessive, yet viability tests indicated these mutants to be 0.9 as viable as the wild type. However, mating tests showed mutant males to be only one-tenth as successful as the wild type so that the rapid decline appears primarily due to selective mating. The more gradual decline in frequency of the sex-linked mutants, yellow and raspberry, which equalled the wild type in viability was again related to mating success. Raspberry males inseminated half as many females, yellow one-tenth as many as the wild type males, and this was reflected in the more rapid decline in frequency of y in the populations. The mode of change conformed to a theoretical partial selection curve, with the most rapid changes occurring at intermediate gene frequencies. Since the wild type became established in 19 of 20 vg and gl populations but only 8 of 19 ras and y populations in which selection pressures were lower, it is clear that chance plays a more important role when selection

pressures are lower. The deviations from a 1:1 sex ratio were greater in the homozygous mutant populations than in those populations in which the wild type became established, a result supporting Fisher's hypothesis that the secondary sex ratio should be adjusted toward unity by natural selection.

The experiments with the sex-linked and autosomal dominant populations are now nearly over, but will not be written up for publication until viability, mating, and possibly other tests have been completed. The sex-linked mutants were Bar and Beadex-3, the autosomals, brown-Dominant, and two different alleles of Lobe. Only bw^D acts as a complete dominant to the wild type, and only in these populations did the wild type have difficulty becoming established from an initial frequency of 0.5% or less. Analysis of the bw^D populations since the last report has shown that they did not carry the recessive wild type in the heterozygous condition. In one population of bw^D, however, the wild type has appeared and started to increase in frequency, indicating that here, too, the wild type is superior, and its difficulties in becoming established must be ascribed to its recessiveness. Contrary to some previous reports by other authors, there has been no indication of an equilibrium between mutant and wild type in any of these populations. Rather, in most populations in which the wild type became established, it has now eliminated the mutant type. Some tests of mating success and viability have been completed since the last report, and these again indicate that the more drastic effects of the mutants are on mating behavior. When the dominants reached a low frequency, they usually were eliminated almost at once from the populations unlike the recessive mutants. This result indicates that selection pressure against the dominants in the populations must have been quite high. These results will be written up for publication when the experiments have been completed.

The results of the experiments with the white series of mutants, white, eosin, apricot, coral and satsuma were also included in the paper "Competition between mutants in experimental populations of Drosophila melanogaster" mentioned above and were summarized in the 1954 Annual Report. The more important points may be briefly mentioned here. These various mutants differed less in selective value than any previously tested, but nevertheless definite trends in gene frequency change were observed in all cases except white vs. satsuma. Hence, even these mutants do not support the concept of the adaptively neutral gene. The sex ratios in all cases showed significant excesses of males, the highest being 2 males: 1 female. Apparently two doses of these mutants in the females were more deleterious than a single dose to the males. Since the eye colors of males and females did not differ greatly, but their viabilities did, Muller's theory of dosage compensation, a system of sex-linked modifying genes adjusting the effects of different doses of the sex-linked genes to comparable values in males and females, does not seem applicable in this case.

Coral and apricot have been shown to be pseudo-alleles of white. Since there was an excess beyond expectation of heterozygous females in those populations, it appears that heterosis may accompany pseudo-allelism, that it is due to the respective wild type alleles

present, and that those cases of allelic interaction reported as supporting the over-dominance theory of heterosis may be due to pseudo-allelism. Since new phenotypes are exposed to natural selection without complete loss of the adaptive value of the wild type, pseudo-allelism may have adaptive value.

The wide deviations in both directions from a 1:1 sex ratio found in the populations with the white series or the autosomal recessive genes indicate that the adaptive values of genes may differ in males and females. This result lends substance to the possibility that balanced polymorphism may be due in some cases to these differences.

An analysis of the second and third chromosomes of Drosophila melanogaster males was made in the fall of 1954. This analysis showed no significant differences between these two large autosomes in the frequency of classes tested. Of the 112 chromosomes analyzed 40.2% were normal, 19.7% sub-vital, 10.7% semi-lethal, and 29.4% lethal. This analysis is to be repeated to determine whether any changes in these frequencies occur and whether they can be related to population size or to environmental factors.

B. Selective mating and sexual isolation

The results of some of the work on selective mating were reported in a paper entitled "Selective mating as a cause of gene frequency changes in laboratory populations of Drosophila melanogaster" published in Evolution 7:287-296(1953). In those experiments, the adverse effects of mutant genes on mating success appeared to be primarily responsible for their decrease in frequency in populations. This same finding has since been made with the other recessive and dominant mutants studied. Since such studies have now been made on about 10 different mutants, it begins to appear that the most deleterious effect of mutant genes is usually on mating behavior and that a large fraction of the selection pressure against such mutants is due to selective mating. Since the Hardy-Weinberg equilibrium, the starting point in population genetics, is based on the assumption of random mating, it is clear that this assumption must be tested. The results seem to indicate that mating behavior, in some respects the most complex physiological mechanism in the organism, is more sensitive to the adverse effects of the mutants than such traits as viability or fecundity. That mutant males are relatively unsuccessful in mating may help account for the virtual absence of mutant phenotypes in most wild populations, well below the expectations based on known gene frequencies and random mating. If selective mating is one of the dominant selective forces, as appears likely, several other possibilities should be noted. On the one hand, selective mating may act as a conservative force in evolution because the more extreme variants in a population are less apt to mate successfully. On the other, favorable mutants can be spread very rapidly through a population due to a mating advantage. Furthermore, differentiation of isolated populations may occur because of differences which develop in their breeding patterns, as distinct from those due to differences in their physical environments, or to genetic drift, or to what Mayr has recently called a change in genetic environment.

A study entitled "Sexual isolation between Drosophila persimilis and Drosophila pseudoobscura" was published in the American Naturalist 88:93-99 (1954). The results of experiments testing the degree of sexual isolation between these closely related species indicated clearly that sexual isolation is determined primarily by the behavior of the females. Sexual isolation was found to be much higher in experiments in which females of one of the species had an opportunity to mate with males of both species than when males of one species had a chance to mate with females of both. This finding narrows down the area of search for the significant factors which lead to a build-up of sexual isolation between populations formerly capable of interbreeding. Since modern species definitions are based on reproductive isolation as the criterion of species distinctiveness, the manner in which such isolation becomes established in evolving populations is a fundamental question.

Further studies of the sexual isolation between these two species are in progress. A fertile strain with wild type eyes has been developed from hybrids between orange D. persimilis and glass D. pseudoobscura, which were back-crossed to D. persimilis. This strain is not sexually isolated from D. persimilis, but is from D. pseudoobscura. Hence, the change may be regarded as essentially a forced introgression of a part of the genotype of D. pseudoobscura, including the wild type allele of orange from D. pseudoobscura, into D. persimilis. An attempt is now under way to break down the sexual isolation between these two species. The hybrid females, which are fertile, are being back-crossed first to males of one species and then to males of the other in the next generation. If this attempt is successful, it may be possible to study the causes of sexual isolation between these species.

C. Experiments on DDT resistance.

The results of some of this work have been submitted for publication to the "Journal of Economic Entomology" and copies of the paper have been forwarded. The results were also summarized in the Annual Progress report for 1954. A brief summary follows:

The development of DDT resistance has been studied in populations derived from inbred, laboratory, and wild stocks of Drosophila melanogaster. Various theories have been proposed to account for the relatively rapid increase in resistance which has occurred in natural populations which have been exposed to DDT. If it is assumed that the mechanism involved is natural selection, then no increase in resistance would be expected in the inbred populations. The fact, therefore, that no increase occurred in the populations derived from the inbred strains during 30 months of selection but an increase has now occurred in all six of the populations derived from wild stocks with a greater store of variability, would seem to bear out the assumption. Only one of seven laboratory stocks developed increased resistance, which would seem to show that the usual methods of maintaining laboratory stocks lead to inbreeding and a reduction in the variability.

Populations derived from hybrids between the inbreds and also from five of the laboratory stocks did not develop increased tolerance on exposure. However, a population descended from five stocks with increased resistance did become more resistant than its control. These results would seem to show that variability per se is not enough but that particular kinds of genes must be present for selection to be effective. Heterosis can probably be ruled out as a major factor in resistance since the controls must have remained at least as heterozygous as the exposed stocks which were under strong selection pressure. Selection was so rigorous at times that as few as 10 surviving adult flies were observed in the exposed populations. Under these circumstances, inbreeding must occur which would tend to reduce the amount of heterosis in the populations.

A further result which seems attributable to the inbreeding resulting from strong selection pressure is the decline in resistance in three of the exposed populations despite continued exposure to DDT. This change was most unusual but was obvious not only from the tests but from the reactions of the exposed populations to DDT concentrations they had previously tolerated. However, inbreeding will bring to expression in the homozygous condition deleterious recessive genes, which will tend to reduce the general viability of the population. Furthermore, with inbreeding, genes favorable to resistance may be lost from the stock by chance. In either event, resistance will decline.

Some preliminary tests indicate that multiple factors are responsible for the increased resistance observed, in agreement with the separate findings of Bochnig, and Crow, and King, also with Drosophila. Unpublished results of other workers in DIS 28 have attributed resistance in Drosophila to a single locus on the second chromosome. Such differences are to be expected. Each population exposed to DDT is unique in its available variability. Selection can act only on whatever variability is present in the population. Hence, if no variability is present, as in the inbreds, or no favorable genes are available, as apparently was the case in some of the hybrid populations, no increase in resistance can occur in response to selection. However, if genes of any kind conferring increased resistance are present in the population, they will tend to increase in frequency. Thus, in some populations, multiple factors, and in others, single dominants might well be expected to be responsible for DDT resistance. Since each population will differ in its initial available variability, and probably in selection pressures other than DDT, in mutation pressures, and in the fluctuations of its population size, it seems almost inevitable that the level of resistance and the genetic mechanism controlling resistance will often differ in different populations. In natural populations, the influx of additional genes through migration will constantly replenish the variability in exposed groups. Thus, the species may show considerable versatility in their ability to adapt to insecticides.

A further result is the finding that the development of resistance was directly related to the strength of selection pressure. Hence, the more effective the control of the population by DDT, the sooner one may expect resistance to evolve. It is rather paradoxical that inbred populations, which failed to become resistant, do not occur in nature, and that the most effective control measures will tend to produce the most resistant populations.

D. Tumor investigation

Mr. Kroman has continued his investigation of a melanotic tumor in Drosophila melanogaster. Some of his results were included in the last Annual Report. The tumor was originally found in an ebony mutant stock, and does not show complete penetrance. However, selection increased the penetrance so that a strain with a penetrance of 85-90% was derived from the original stock with penetrance half as great.

The tumor is due to major genes on the second and third chromosomes. The gene on II is semi-dominant and found between 0 and 10 units; the gene or genes on III are recessive.

Since the melanotic tumors appeared in the ebony stock, it was suspected that ebony itself, which increases melanin production, might play a role in the tumor formation. However, crosses of the e^{11} tumor stock to other ebony stock gave no tumors in the F_1 desoite being ebony in phenotype and having the semi-dominant factor on II present. Substitution of a sex chromosome bearing yellow in the e^{11} tumor stock gave a yellow-ebony tumor stock phenotypically lighter than the wild type. However, in this case the tumor incidence remained high. Hence, it appears that melanin production controlled by ebony is not directly involved in tumor formation. An attempt to introduce black into the tumor stock since it too increases melanin production resulted in the loss of tumors. Hence, it appears that the melanin production controlled by the ebony and black loci do not determine the appearance of the melanotic tumors. On the other hand, in the tumor stock, it has thus far not been possible to obtain a high frequency line which lacks ebony. Occasional non-ebony tumor flies have appeared in the crosses, but selection has been ineffective in prpducing a non-ebony tumor line.

A report that heat shock intensifies the amount of pigment in heterozygous ebony flies was used as the basis for experiments to raise tumor incidence in flies homozygous for the 2nd chromosome tumor factor and heterozygous for ebony. No increase in melanin production and no tumors were observed. Crosses of the ebony tumor stock to a wild strain, Oregon R, did result in occasional tumors. Presumably, some tumor factors must be present in this wild strain.

Among environmental effects, the higher the temperature at which the flies were reared, the lower the frequency of tumors.

A study of the effect of maternal age on tumor incidence has shown that there was no significant decrease in tumor frequency over a 10-day period. When tumor incidence was measured among flies which emerged over a period of time from the same food bottle, it was found that the frequency was higher among the last flies to emerge.

At present certain facts are clear. The tumors are under genetic control, the result of the interaction of at least two major genes on two different chromosomes. Modifiers are involved also since selection raised the tumor incidence. Ebony^{11} or genes closely linked to it are implicated, since a non-ebony tumor stock has not been obtained. However, substitution of other ebony alleles results in the absence of tumors. The environment plays a role in the tumor incidence, both temperature and nutrition affecting tumor frequency.

LIST OF REPORTS AND PUBLICATIONS:

Merrell, David J. 1953. Gene frequency changes in small laboratory populations of Drosophila melanogaster. Evolution 7:95-101.

_____ 1953. Selective mating as a cause of gene frequency changes in laboratory populations of Drosophila melanogaster. Evolution 7:287-296.

_____ 1954. Sexual isolation between Drosophila persimilis and Drosophila pseudoobscura. American Naturalist 88:93-99.

_____ and James C. Underhill. Competition between mutants in experimental populations of Drosophila melanogaster. Submitted to Evolution.

_____ Selection for DDT resistance in inbred laboratory, and wild stocks of Drosophila melanogaster. Submitted to the Journal of Economic Entomology.

Finally, I wish to express my appreciation for the support received from the Office of Naval Research. It has made it possible for me to carry on more research than otherwise would have been possible with my present teaching load, and came at a most helpful time. In addition, it has provided experience and support to several graduate students, primarily Mr. Underhill and Mr. Kroman.

Respectfully submitted,

David J. Merrell